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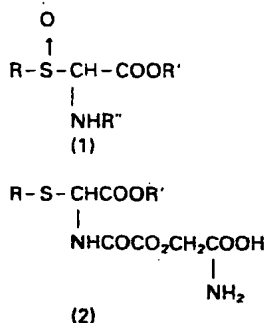
⑥④ Flavor enhancing seasonings and foods containing them.

⑤⑦ The invention relates to seasoning and foods comprising
i) a taste intensifying substance, and
ii) a flavor enhancing substance which is at least one
member selected from glutathione, a salt of glutathione,
oxidized glutathione, a salt of oxidized glutathione sulfonic
acid, cycloallin, a salt of cycloallin, lentinic acid, a salt of
lentinic acid, des-glutamyl lentinic acid, a salt of des-
glutamyl lentinic acid, methionine methyl sulfonium, a salt of
methionine methyl sulfonium, a compound of the formulae

wherein R is a saturated or unsaturated aliphatic hydrocar-
bon group of from 1 to 3 carbon atoms or a carboxyalkyl
group wherein said alkyl is from 1 to 6 carbon atoms,

R' is H, a γ -glutamyl group or a salt of a γ -glutamyl
group,

a salt of compound (1) and a salt of compound (2).



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FLAVOR ENHANCING SEASONINGS AND
FOODS CONTAINING THEM

This invention relates to seasoning and food having enhanced flavor by the addition of sulfur-containing compounds.

The taste intensity imparting function of representative "Umami" substances, namely, sodium L-glutamate (MSG), sodium 5'-inosinate (IMP) and sodium 5'-guanylate (GMP) is widely known. A wide variety of seasonings obtained from MSG, IMP and GMP can be appropriately combined with protein hydrolysates (HVP, HAP), yeast extract (YE), amino acids etc. In their various application, these substances have been increasingly used for the purpose of enhancing organoleptic characteristics of foods such as the "Umami" (MSG like taste) flavor etc.

Although "Umami" substances enjoy popularity and wide application, there has still been a demand for

extending their function; to further develop their flavor characteristics. More specifically, there has still been a demand to further develop the thickness, amplitude, continuity etc. of "Umami" substances. This is a property which is different from their taste which is the effect achieved by a combination of saltiness, sweetness, acid taste etc. as is done to further enhance a taste.

Hence, throughout this text the term taste is used to describe the basic saltiness, sweetness and acidity properties of the "Umami" substances described above. The term flavor is used to describe the thickness, amplitude and continuity properties of "Umami" substances. The terms taste and flavor do not describe the same properties. Rather they describe different properties which can be complementary.

SUMMARY OF THE INVENTION

Accordingly, one object of this invention is to provide a novel enhanced flavor seasoning and foods containing it.

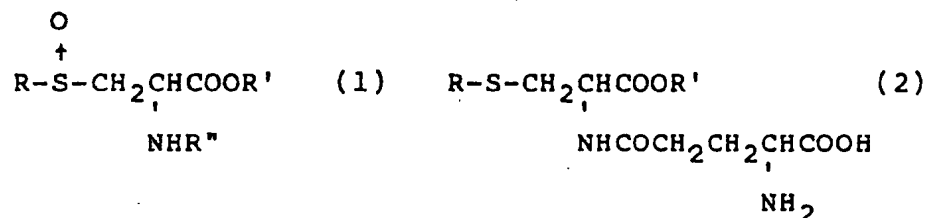
The present inventors have discovered that

- (1) glutathione (GSH) and oxidized glutathione (GSSG) -- hereinafter called generically as "GSH" --,
- (2) glutathione sulfonic acid (GSO_3H), (3) cycloallin -- hereinafter called "CA" --, (4) lentinic acid,

des-glutamyl lentinic acid and the salts thereof --

hereinafter called generically as "LA" --,

(5) methionine methyl sulfonium (MMS), and (6) compound of the following general formula (1) or (2):



wherein R is a saturated or unsaturated aliphatic hydrocarbon group of 1-3 carbon atoms or a carboxyalkyl group wherein the alkyl group is from C₁ to C₆,

R' is H or a carboxymethylamino group, and

R'' is H or a γ-glutamyl group,

have flavor intensifying properties.

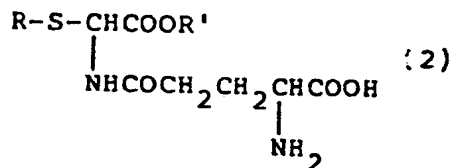
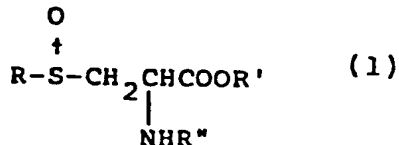
As the result of further detailed investigations on taste and flavor intensity, it has been discovered that these compounds manifest strong flavor enhancement in the presence of MSG, a nucleic acid type taste intensifying substance or a mixture thereof.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Glutathione (GSH), oxidized glutathione (GSSG), glutathione sulfonic acid (GSO₃H), cycloallin (CA), lentinic acid and des-glutamyl lentinic acid (both hereinafter generically referred to as LA) and methionine methyl sulfonium (MMS) have been confirmed to be widely present in foods, natural products etc.

Part of compounds (1) and (2) shown above were known as precursor substances for specific taste components in food in which these are present. Other parts of compounds (1) and (2) are presumed to be present in the metabolism route in foods, but no reference has been made to their relation to flavor intensity. Further, it is impossible to guess what flavor intensifying function these compounds manifest in the presence of the Umami components.

It has now been discovered that GSH, GSSG, GSO₃H, CA, LA, MMS and compounds of the general formulae (1) and (2) shown below



wherein R is a saturated or unsaturated aliphatic hydrocarbon group of 1 to 3 carbon atoms or a carboxyalkyl group wherein the alkyl group is from C₁ to C₆,

R' is H or a carboxymethylamino group, and

R'' is H or a γ-glutamyl group

manifest strong flavor enhancement in the presence of MSG, a nucleic acid type taste intensifying substance or a mixture thereof. As the result of a detailed

study on various combinations and formulations of glutamic acid, glutamic acid salts or nucleic acid type taste intensifying substances with "CA", "GSH", "GSO₃H", "LA" or "MMS", it has been discovered that these sulfur-containing compounds are widely effective in the enhancement of flavor. Their discovery thus provides the possibility for novel flavor enhancing seasonings and foods by incorporating together "Umami" components and the sulfur-containing compounds. The present invention relates to such combinations.

GSH has been studied for its physiological activity, pharmacological efficacy etc. It has been used as e.g. a liver disease treating agent, an antipyretic etc. Various applications of GSH to foods are also known. Some of these applications are given below. (1) GSH has been added to dough products of e.g., flour etc. as the so-called amino acid type, reducing agent like cysteine etc. (See Japanese Patent Publication Nos. 19863/1974, 16418/1975, 1773/1976, 9019/1976, 33981/1976, 9893/1981, Japanese Patent Application Laid-open Nos. 11967/1972, 30855/1972, 98052/1973, 122762/1979, 23853/1981 etc.) (2) Similarly, GSH has been added to sour milk and baby foods as a storage stabilizer for vitamin C. (See Japanese Patent Publication Nos. 7143/1975 and 21606/1970.) (3) GSH has been added to fish paste

products such as boiled fish taste as a decomposition inhibitor for nucleic acid type taste intensifying substances. (See Japanese Patent Publication No. 8694/1968.) (4) GSH has been added as an agent to relieve the yeast odor of yeast extract. (See Japanese Patent Publication No. 6232/1969.) (5) GSH has been used for the purpose of preventing the discoloration of canned fruit. (See Japanese Patent Publication No. 20655/1965.) (6) It has been used to intensify the flavor of foods, especially canned meat by being combined with sugar, amino acids etc. by reaction. (See Japanese Patent Publication Nos. 22194/1967, 35149/1974, 28977/1978 etc.) (7) It has been used to intensify cheese flavor. (See Japanese Patent Publication No. 25024/1978 and the like.)

In these examples of applications of GSH to foods, GSH is used as a physical property modifier as in above (1). In (4), (6) and (7) etc. above, there are no comments regarding the taste intensity of GSH. Furthermore, the amounts of GSH added are only extremely small amounts. For example, only 0.01 to 0.05% GSH is added in the case of dough products, 0.001 to 0.02% in the case of baby foods. Even in the case of taste intensification in meats or cheeses (as in (6) or (7) above), a similarly extremely small amount is added. Therefore, it is clear from the

above examples that there has been no finding in the prior art of GSH actually having a flavor intensifying function. Moreover, it is entirely impossible to know or predict what flavor intensifying function, if any, GSH would manifest in the presence of other taste components.

GSO₃H is known as a component in enokidake. Its presence has also been confirmed in a part of other mushrooms. The significance of its presence or its role in food have not yet been described. There is no reference in the prior art concerning the taste intensity of GSO₃H. Further it is entirely impossible to know or predict what flavor intensifying functions, if any, GSO₃H manifests in the presence of the "Umami" components.

Lentinic acid is contained in shiitake and is known as a precursor to the odor of shiitake. When shiitake tissue is damaged or dried shiitake is reconstituted with water, it undergoes various chemical and/or biochemical reactions; e.g., enzymatic action by γ -glutamyl transferase, C-S lyase etc. Resultingly lenthionine which is an odorous principle of shiitake is formed.

In none of the above-described research was there any mention suggesting the flavor intensity of lentinic

acid. Further, it is entirely impossible to know or predict what flavor intensifying functions, if any, lentinic acid manifests in the presence of the "Umami" components.

MMS is also known as vitamin U. It has been intensively investigated for its pharmacological efficacy and has been used as e.g. an antiulcer agent. Various applications of MMS in foods are also known. Some of these applications are given below.

(1) MMS has been used also for the purpose of intensifying and modifying the taste of food by adding it as a so-called taste precursor substance to the food and subsequently converting it into a taste substance by heating. (See Japanese Patent Application Laid-open No. 92663/1979 etc.) (2) MMS has been used for the purpose of enhancing the nutritional value of soybean paste, bean milk etc. (See Japanese Patent Publication Nos. 19827/1968 and 13979/1974.) (3) It is used as an antioxidant for oils and fats. (See Japanese Patent Publication No. 1125/1975.) (4) And it is used for the purpose of preventing the decomposition of vitamin B₁. In this use it is incorporated in compositions to which sulfurous acid has been added to serve as a deterioration inhibitor; e.g., medicines, foods etc. (See Japanese Patent Publication No. 6153/1963.)

There has been no disclosure relating to the flavor intensity of MMS in any of these application. Further, it is entirely impossible to guess or predict what flavor intensifying function, if any, MMS manifests in the presence of the "Umami" components.

Cycloallin is also known as imino acid. It is a sulfur-containing component in onion and garlic. There are only few studies of its characteristics. It is known that cycloallin is formed by reaction of s-propenylcysteinsulfoxide which is contained in 3% by wt. as a dry matter in onion. In none of the research relating to cycloallin, described above, are there any remarks of the flavor intensity of cycloallin. Further, it is entirely impossible to know or predict what flavor intensifying functions, if any, cycloallin manifests in the presence of the "Umami" components.

In accordance with the present invention, a characteristic flavor intensity enhancing effect can be obtained by using "GSH", GSO_3H , "CA", "LA" and/or MMS in combination with conventionally known "Umami" compounds such as MSG, IMP, GMP etc. The present invention further provides that these combinations are made in accordance with specified ratios. This invention relates to the intensification of the impression of taste (flavor enhancement) and to further

impart flavor. Flavor here is the continuity, amplitude, thickness, etc. of the original taste "Umami", bitterness, sweetness, acid taste, saltiness etc. while the original taste remains unchanged.

The mixing ratio of the "Umami" component to the sulfur-containing component selected from the group consisting of "GSH", GSO_3H , "CA", "LA" and MMS may be determined as follows. The amount of the sulfur-containing compound or compounds added is within the range of 0.1 to 1000% by weight based on the weight of the present "Umami" component. The "Umami" component is calculated as the weight of MSG required to manifest the same "Umami" strength. All comparisons are made to the "Umami" strength of MSG. When a nucleic acid type taste intensifying substance such as IMP, GMP etc. is used alone, the mixing ratio of sulfur-containing compound or compounds is 1 : 99 to 99 : 1. For example, in the case where the "Umami" component comprises only MSG, the sulfur-containing compound or compounds are present at a concentration of 0.001 to 10 times that of MSG. In the other case, i.e. where MSG and IMP or GMP are also present as the "Umami" components, the "Umami" strength (y value) may be determined according to the following equations:

$$\text{IMP: } y = u + 1218 \text{ uv}$$

$$\text{GMP: } y = u + 2800 \text{ uv}$$

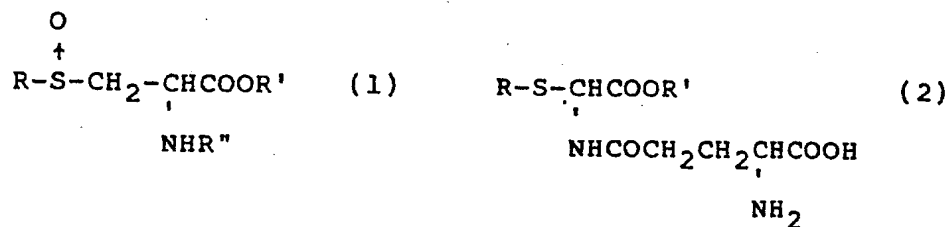
wherein u = MSG concentration (%), and v = IMP or GMP concentration (%).

While the conversion equation to obtain each y value can vary depending on the kinds and number of the tastiness components etc., the conversion equation established by organoleptically comparison with a simple aqueous solution of MSG may be appropriately used. For instance, the y value where IMP and GMP are used in combination at a ratio of 50:50 is as follows:

$$y = u + 2010 uv$$

wherein u = MSG concentration (%), and v = IMP/GMP mixture concentration (%).

In this invention a characteristic flavor enhancing effect can also be obtained by using the compounds of the general formulae (1) and (2) (shown below) either singly or as a mixture.



wherein R is a saturated or unsaturated aliphatic hydrocarbon group of 1 to 3 carbon atoms, or a carboxyalkyl group wherein the alkyl group is from C_1 to C_6 ;

R' is H or a carboxymethylamino group, and

R" is H or a γ -glutamyl group.

The compounds of general formulae (1) and (2) are used in an amount falling in a specified ratio to the "Umami" components such as MSG, IMP, GMP etc. The effect in this case is to intensify the impression of the taste and further impart flavor; to further develop the continuity, amplitude, thickness etc. The flavor thus obtained is characterized by that the original taste -- "Umami" bitterness, sweetness, acid taste, saltiness etc. -- remains unchanged.

The mixing ratio of the "Umami" component to these compounds may be determined as follows. The amount of compound (1) or (2) or combination thereof added is 0.1 to 1000% by weight based on the weight of the also present "Umami" component. The weight of the also present "Umami" component is calculated as the weight of, e.g. MSG, required to manifest the same "Umami" strength. When a nucleic acid type taste intensifying substance such as IMP, GMP etc. are used alone, the mixing ratio of these to compounds (1) or (2) or combination thereof is in the range of 1:99 to 99:1. In other words, in the case where the "Umami" component comprises only MSG, compound (1) or (2) or combination thereof are present in an amount of 0.001 to 10 times the concentration of MSG. In the other case, i.e.,

where MSG and IMP or GMP are present as the "Umami" components, the "Umami" strength (y value) may be determined according to the following equations:

$$\text{IMP: } y = u + 1218 uv$$

$$\text{GMP: } y = u + 2800 uv$$

wherein u = MSG concentration (%), and v = IMP or GMP concentration (%).

While the conversion equation to obtain each y value can vary depending on the kinds and number of the "Umami" components etc., y values established by organoleptic comparison against a simple aqueous solution of MSG may be appropriately used. For instance, the y value where IMP and GMP are used in combination at a ratio of 50:50 is as follows:

$$y = u + 2010 uv$$

wherein u = MSG concentration (%), and v = IMP/GMP mixture concentration (%).

The compounds of formulae (1) and (2) can also be used in combination with any of the other sulfur-containing compounds: "GSH", GSO_3H , "CA", "LA" and MMS.

Other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments which are given for illustration

of the invention and are not intended to be limiting thereof.

Experimental Example 1

(Evaluation in Simple Aqueous Solution Systems)

Using as a control a mixed aqueous solution of 0.05 g/dl of MSG and 0.05 g/dl of IMP (y value = 3.09), sample solutions were prepared by adding 0.001 to 0.5 g/dl of GSH to the mixed aqueous solution. These sample solutions were organoleptically evaluated by paired comparison tests against the control using an organoleptic panel of 20 members ($N = 20$). The results are given in Table 1.

Table 1

$N = 20$

	<u>Formulation</u>			<u>Taste Intensifying Effect</u>	
	<u>MSG g/dl</u>	<u>IMP g/dl</u>	<u>GSH g/dl</u>	<u>Strength of "Umami"</u>	<u>Strength of Flavor Enhancement</u>
Control	0.05	0.05	--	±	±
Sample A	"	"	0.001	±	±
B	"	"	0.01	±	++
C	"	"	0.05	±	+++
D	"	"	0.1	+	++++
E	"	"	0.3	±	+++++
F	"	"	0.5	±	+++++

The following notation is used throughout the examples: (+) = enhancement of property being investigated, (++) = pronounced enhancement, etc., (+) = no noticeable enhancement, (-) = mitigation of property being investigated.)

As shown in Table 1, with less than 0.1% of GSH added, based on the y value (MSG calculated amount), the strength of flavor is comparable to that of the control. But by the addition of 0.1% or more of GSH, the flavor alone is remarkably increased without changing the strength of "Umami" and other basic tastes. If the absolute concentration of GSH exceeds 1.0%, specific thickness in taste is manifested and the total taste balance is lost. Therefore, the amount of GSH added should suitably be in the range of 0.1 to 1000% by weight, preferably 0.1 to 50% by weight, based on the weight of the also present tastiness component. Where the amount of tastiness component used is calculated as the weight of MSG having the same "Umami" strength. (See equations above.) Further, GSH manifests the most preferred effect in the presence of an appropriate amount of the above "Umami" component, that is, when the above y value is 0.1 to 30 as the concentration at the time of eating.

On the other hand, in the case where the nucleic acid type taste intensifying substance such as IMP, GMP etc. is used alone, if GSH is added at a ratio of less than 1/99 to the nucleic acid type taste intensifying substance, the flavor enhancing effect is not adequate. Further when GSH is present in an amount of 250%/wt. or more based on the amount of nucleic acid type taste intensifying substance, GSH manifests the most preferred effect. Whereas if GSH exceeds a ratio of 99/1, a specific thickness in taste is brought about and the total taste balance is lost.

Experimental Example 2

(Evaluation in Beef Extract Systems)

According to the analytical values of beef extract, the effect of the addition of GSH to a formulation of major components was evaluated by paired comparison test against a control. The results are given in Table 3, from which it has been confirmed that in the synthetic beef extract as well, GSH imparts a higher natural taste by manifesting a strong flavor enhancement.

Table 2
Formulation of Major
Components of Beef Extract

	<u>Formulation</u>
MSG	3 %
IMP	3
NaCl	11
K ₂ HPO ₄	10
KH ₂ PO ₄	25
Sodium lactate	20
Histidine hydrochloride	20
Alanine	4
Lysine hydrochloride	4
	100

Table 3

	<u>Major Components g/dl</u>	<u>GSH g/dl</u>	<u>Strength of Flavor Enhancement</u>
Control	2	0	±
Sample A	"	0.01	++
B	"	0.1	++++
C	"	0.2	+++

GSH in this invention may be obtained by any production process, such as an extraction process, a synthesis process etc. Further, it is also possible to replace the whole or part of the required GSH by a substance containing GSH.

Experimental Example 3
(Evaluation in Shrimp Extract Systems)

According to the analytical values of the extract components of shrimp which is a natural delicious food, the major components were formulated and organoleptically evaluated similarly as in Experiment Example 2. These tests confirm, as shown in Table 5, that GSH imparted a higher natural taste by manifesting a strong flavor enhancement as it did in beef extract tests.

Table 4
 (Formulation of Major Components)

MSG	3.9 %
IMP	3.9
NaCl	12.6
K ₂ HPO ₄	13.0
KH ₂ PO ₄	5.3
Sodium lactate	15.3
Alanine	15.4
Glycine	15.3
Arginine	<u>15.3</u>
	100.0

Table 5

	Major Component (g/dl)	GSH (g/dl)	Strength of Flavor Enhancement	Strength of Natural Feel
Control	1.3	0	±	±
Sample A	"	0.006	++	+++
B	"	0.06	++++	++++
C	"	0.12	+++++	+++

Experimental Example 4
(Evaluation in Simple Aqueous Solution Systems)

A mixed aqueous solution of 0.05 g/dl of MSG and 0.05 g/dl of IMP (γ value = 3.09) was used as a control. Sample solutions were prepared by adding 0.002 to 1.0 g/dl of GSO_3H to the mixed aqueous solution, and these were organoleptically evaluated by paired comparison test against the control using an organoleptic panel of 20 members ($N = 20$). The results are given in Table 6.

Table 6

				N = 20	
	<u>Formulation</u>			<u>Taste Intensifying Effect</u>	
	<u>MSG g/dl</u>	<u>IMP g/dl</u>	<u>GSO_3H g/dl</u>	<u>Strength of "Umami"</u>	<u>Strength of Flavor Enhancement</u>
Control	0.05	0.05	--	±	±
Sample A	"	"	0.002	±	±
B	"	"	0.02	±	+
C	"	"	0.05	±	+
D	"	"	0.1	±	++
E	"	"	0.15	±	+++
F	"	"	0.5	±	+++
F'	"	"	1.0	±	+++
G	--	0.1	0.1	-	++

As shown in Table 6, with less than 0.1% of GSO_3H added, based on the γ value (MSG calculated amount), the strength of flavor enhancement is comparable to

that of the control. By the addition of 0.1% or more, the flavor enhancement alone is remarkably increased without changing the strength of "Umami" component. If the absolute concentration of GSO_3H exceeds 1.0%, specific thickness in taste is manifested and the total taste balance is lost. Therefore, the amount of GSO_3H added should suitably be in the range of 0.1 to 1000% by weight, preferably 0.1 to 50% by weight, based on the weight of co-present "Umami" component. The amount of "Umami" component used is calculated as the weight of MSG having the same "Umami" strength. (See equations.) Further, GSO_3H manifests the most preferred effect in the presence of an appropriate amount of the above "Umami" component, that is, when the above y value is 0.1 to 30 as the concentration at the time of eating.

On the other hand, if the nucleic acid type taste intensifying substance is used alone and GSO_3H is added at a ratio of less than 1/99 relative to the nucleic acid type taste intensifying substance, the flavor enhancing effect is not adequate. Whereas if this ratio exceeds 99/1, a specific thickness in taste is brought about and the total taste balance is lost.

Experimental Example 5
(Evaluation in Simple Aqueous Solution Systems)

A mixed aqueous solution of 0.05 g/dl of MSG and 0.05 g/dl of IMP (γ value = 3.09) was used as a control. Sample solutions were prepared by adding 0.002 to 1.0 g/dl of lentinic acid or des-glutamyl lentinic acid (LA) to the mixed aqueous solution. These sample solutions were organoleptically evaluated by paired comparison against the control using an organoleptic panel of 20 members (N = 20). The results are given in Table 7.

Table 7

	<u>Formulation</u>			<u>Taste Intenstifying Effect</u>	
	<u>MSG</u> <u>g/dl</u>	<u>IMP</u> <u>g/dl</u>	<u>"LA"</u> <u>g/dl</u>	<u>Strength</u> <u>of "Umami"</u>	<u>Strength of</u> <u>Flavor</u> <u>Enhancement</u>
Control	0.05	0.05	--	±	±
Sample A	"	"	0.002	±	±
B	"	"	0.02	±	+
C	"	"	0.05	±	+
D	"	"	0.1	±	++
E	"	"	0.5	±	+++
F	"	"	1.0	±	+++
G	--	0.1	0.1	-	++

As shown in Table 7, with amounts of added lentinic acid or des-glutamyl lentinic acid less than 0.1% based on the y value (MSG calculated amount), the strength of flavor enhancement is comparable to that of the control. But by the addition of 0.1% or more of lentinic acid or des-glutamyl lentinic acid, the flavor enhancement alone is remarkably increased without changing the strength of "Umami". If the absolute concentration of lentinic acid or des-glutamyl lentinic acid exceeds 1.0%, specific thickness in taste is manifested and the total taste balance is lost. Therefore, the amount of "LA" added should suitably be in the range of 0.1 to 1000% by weight, preferably 0.1 to 50% by weight, based on the weight of co-present tastiness component calculated as the weight of MSG having the same "Umami" strength. Further, "LA" manifests the most preferred effect in the presence of an appropriate amount of the above "Umami" component. That is, when the above y value is 0.1 to 30 as the concentration at the time of eating.

On the other hand, in the case where a nucleic acid type taste intensifying substance such as IMP, GMP etc. is used alone, if "LA" is added at a ratio of less than 1/99 to the nucleic acid type taste intensifying substance, the flavor enhancing effect is not

adequate. Whereas if this ratio exceeds 99/1, a specific thickness in taste is brought about and the total taste balance is lost.

Experimental Example 6
(Evaluation in Simple Aqueous Solution Systems)

A mixed aqueous solution of 0.05 g/dl of MSG and 0.05 g/dl of IMP (y value = 3.09) was used as a control. Sample solutions were prepared (1) by adding 0.001 to 1.0 g/dl of MMS to the mixed aqueous solution, and (2) by mixing aqueous solutions of 0.1 g/dl of IMP and 0.1 g/dl of MMS. These sample solutions were organoleptically evaluated by paired comparison test against the control using an organoleptic panel of 20 members (N = 20). The results are given in Table 8.

Table 8

N = 20

	<u>Formulation</u>			<u>Taste Intenstifying Effect</u>	
	<u>MSG</u> <u>g/dl</u>	<u>IMP</u> <u>g/dl</u>	<u>MMS</u> <u>g/dl</u>	<u>Strength</u> <u>of "Umami"</u>	<u>Strength of</u> <u>Flavor</u> <u>Enhancement</u>
Control	0.05	0.05	--	±	±
Sample A	"	"	0.002	±	±
B	"	"	0.01	±	+
C	"	"	0.05	±	++
D	"	"	0.1	±	++++
E	"	"	0.5	±	+++++
F	"	"	1.0	±	++++
H	---	0.1	0.1	-	++++

As shown in Table 8, when the amount of added MMS is less than 0.1% based on the y value (MSG calculated amount), the strength of flavor enhancement and the characteristic sweetness are comparable to that of the control. But by the addition of 0.1% or more, the flavor enhancement and the characteristic sweetness are remarkably increased without changing the strength of "Umami" and the characteristic sweetness. If the concentration of MMS exceeds 1.0%, a characteristic aroma or specific thickness in taste is manifested and the total taste balance is lost. Therefore, the amount of MMS added should suitably be in the range of 0.1 to 1000% by weight, preferably 0.1 to 50% by weight, based on the weight of also present "Umami" component. The amount of "Umami" component used is calculated as the weight of MSG having the same "Umami" strength. (See equations.) Further, MMS manifests the most preferred effect in the presence of an appropriate amount of the above "Umami" component. That is, when the above y value is 0.1 to 30 as the concentration at the time of eating.

On the other hand, when a nucleic acid type taste intensifying substance such as IMP, GMP etc. is used alone, if MMS is added at a ratio of less than 1/99 to the nucleic acid type taste intensifying substance, the flavor enhancement as well as the characteristic

sweetness enhancing effect is not adequate. Whereas if the ratio exceeds 99/1, a characteristic sweetness or specific thickness in taste is brought about and the total taste balance is lost.

Experimental Example 7
(Evaluation in Onion Extract Systems)

According to the analytical values of the onion extract, the effect of the addition of MMS to the formulation of major components was evaluated by a paired comparison test against a control case. The results are given in Table 10, from which it has been found that MMS imparts clear vegetable characteristic sweetness and strong flavor enhancement and gives a natural feel.

Table 9
(Formulation of Major
Components in Onion Extract)

	<u>Formulation (%)</u>
Glucose	25
Sucrose	15
Maltose	25
Amino Acids *	10
Na Citrate	15
Na Malate	<u>10</u>
	100

* Amino acids: a mixture of MSG, arginine, lysine, isoleucine, leucine, aspartic acid, glycine, phenylalanine and proline.

Table 10

	<u>Major Component</u>	<u>MMS</u>	<u>Charac- teristic Sweetness</u>	<u>Flavor Enhance- ment</u>	<u>Natural Feel</u>
Control	2 g/dl	--	±	±	-
Sample A	"	0.01 g/dl	+	++	±
Sample B	"	0.1	++	+++	+
Sample C	"	0.2	++	++++	++

(ca. 0.04 g/dl as an MSG concentration)

Experimental Example 8

(Evaluation in Simple Aqueous Solution Systems)

A mixed aqueous solution of 0.05 g/dl of MSG and 0.05 g/dl of IMP (γ value = 3.09) was used as a control. Sample solutions were prepared by (1) adding 0.002 to 1.0 g/dl of cycloallin to the mixed aqueous solution and (2) by adding 0.1 g/dl of cycloallin to an aqueous solution of 0.1 g/dl of IMP. These sample solutions were organoleptically evaluated by paired comparison tests against the control using an organoleptic panel of 20 members (N = 20). The results are given in Table 11.

Table 11

N = 20

	<u>Formulation</u>			<u>Taste Intensifying Effect</u>	
	<u>MSG</u> <u>g/dl</u>	<u>IMP</u> <u>g/dl</u>	<u>Cyclo-</u> <u>Alliin</u> <u>g/dl</u>	<u>Strength</u> <u>of "Umami"</u>	<u>Strength of</u> <u>Flavor</u> <u>Enhancement</u>
Control	0.05	0.05	--	±	±
Sample A	"	"	0.002	±	±
B	"	"	0.02	±	+
C	"	"	0.05	±	+
D	"	"	0.1	±	++
E	"	"	0.5	±	+++
F	"	"	1.0	±	+++
G	--	0.1	0.1	±	+

As shown in Table 11, when the amount of added cycloallin is less than 0.1% based on the y value (MSG calculated amount), the strength of flavor enhancement is comparable to that of the control. But by the addition of 0.1% or more, the flavor enhancement alone is remarkably increased without changing the strength of "Umami". If the absolute concentration of cycloallin exceeds 2.0%, specific thickness in taste is manifested and the total taste balance is lost. Therefore, the amount of allin added should suitably be in the range of 0.1 to 1000% by weight, preferably 0.1 to 50% by weight, based on the weight of co-present "Umami" component calculated as the weight of MSG

having the same "Umami" strength. Further, cycloallin manifests the most preferred effect in the presence of an appropriate amount of the above "Umami" component. That is, when the above y value is 0.1 to 30 as the concentration at the time of eating.

On the other hand, when a nucleic acid type taste intensifying substance is used alone, if cycloallin is added at a ratio of less than 1/99 to the nucleic acid type taste intensifying substance, the flavor enhancing effect is not adequate. Whereas if the ratio exceeds 99/1, specific thickness in taste is brought about and the total taste balance is lost.

GSH or GSO_3H in this invention may be that produced by any process, e.g., a fermentation process, an enzymatic process, a chemical synthetic process or mixtures thereof, etc. Further, it is also possible to replace the whole or part of the amount of the required GSH by a substance containing GSH at a relatively high concentration such as yeast extract, garlic extract, beef extract, chicken extract etc. The same may be done with GSO_3H where a substance containing GSO_3H at a relatively high concentration, such as enokidake extract, is used.

Lentinic acid in this invention may be obtained from shiitake as is well known in the art, e.g. by

extraction with alcohol, extraction with hot water, etc. Further, it is also possible to replace the whole or part of lentinic acid by an intermediate obtained by such extraction.

Cycloallin in this invention may be obtained from onion or garlic as is well known in the art, e.g. by extraction with alcohol, extraction with hot water, etc. Further, it is also possible to replace the whole or part of cycloallin by an intermediate, end product, or a chemical or enzymatic-reaction product of the extract containing the precursor.

Similarly, as regards the glutamic acid, nucleic acid type taste intensifying substances and other "Umami" components, the whole or part of said "Umami" components may be replaced by such "Umami" components as contained in foods and seasonings per se to which the sulfur-containing compound or compounds are to be added.

The "Umami" components used in combination with the sulfur-containing compounds in this invention include, in addition to MSG, IMP and GMP, any component having a "Umami" imparting effect. For example, potassium glutamate, calcium glutamate, potassium 5'-inosinate, calcium 5'-inosinate, potassium 5'-guanylate, calcium 5'-guanylate etc. and any food or

seasoning which contains at least one tastiness component as one of its constituents. For example, HAP, HVP, yeast extract, meat extract, fish extract, vegetable extract etc. may be used.

The flavor enhancement seasonings of this invention may be prepared by any process. For example, the seasonings may be prepared by merely dry blending MSG, IMP, GMP etc. with these compounds by pelletizing them together with an appropriate excipients, etc. Similarly, with regards to the production of food having enhanced flavor in accordance with this invention, the actual manner for the production is not limited as long as the "Umami" compounds and the sulfur-containing compound or compounds are co-present in the final product at the above-described mixing ratio.

In addition, it is needless to say that the use in combination of various seasonings, for example, salty taste agents such as table salt, potassium chloride etc., sweetening agents such as sugar etc., acid taste agents such as organic acids etc., flavors, spices, physical property modifiers etc. is possible unless it departs from the object of this invention.

The seasoning or food of this invention manifests a strong tastiness enhancement as is the case when

seasoned with e.g. MSG, IMP, GMP etc. The quality of these seasonings is refreshing but, differing from the seasoning effect characterized by such strong single taste as simple taste. This quality exerts a specific effect permitting the enhancement of only the flavor without changing the "Umami" strength and the original taste such as saltiness, sweetness and the like. The seasoning or food of the present invention are characterized by the impartation of a complex natural taste. Therefore the seasonings of the present invention provide a novel flavor enhancement seasoning having a high natural taste in the compound seasoning field.

This invention is more particularly described by the following examples.

Example 1

Formulation of Flavor Enhancement Seasoning A

MSG	80 parts by weight
GSH	20 "

Formulation of Flavor Enhancement Seasoning B

MSG	75 parts by weight
IMP	5 "
GSH	20 "

Formulation of Flavor Enhancement Seasoning C

MSG	50 parts by weight
GMP	10 "
GSH	40 "

Formulation of Flavor Enhancement Seasoning D

MSG	80 parts by weight
IMP	3 "
GMP	3 "
GSH	14 "

Formulation of Flavor Enhancement Seasoning D₁

IMP	30 parts by weight
GMP	30 "
GSH	40 "

Flavor Enhancement Seasonings A to D₁ were prepared by mixing the respective compounds according to the above formulations. Using as each control a product to which GSH had not been added, aqueous solutions of 0.4 g/dl of the respective samples were prepared, and organoleptically evaluated by a paired comparison test using a 20 member panel of judges. The results are given in Table 12.

Table 12

N = 20

	<u>A</u>	<u>B</u>	<u>C</u>	<u>D</u>	<u>D_T</u>
Strength of "Umami"	+	++	++++	+++	±
Strength of Flavor Enhancement	+++	+++++	++++++	++++++	+++++

Example 2

Table 13

Formulations of Flavor
Enhancement Seasonings E - H

(parts by weight)

	<u>E</u>	<u>F</u>	<u>G</u>	<u>H</u>
HVP	60	60	60	60
MSG	26	26	26	26
IMP	1	1	1	1
GMP	1	1	1	1
2Na Succinate	2	2	2	2
NaCl	10	10	10	10
GSH	1.7	8.3	16.7	33.3

Flavor enhancement Seasonings E to H were prepared by mixing the respective components according to the above formulations. Using as each control a product to which GSH had not been added, organoleptic evaluation was conducted by a paired comparison test similarly as in Example 1. The results are given in Table 14.

Table 14

	N = 10				
	<u>Control</u>	<u>E</u>	<u>F</u>	<u>G</u>	<u>H</u>
Concentration (Simple Soln) (g/dl)	0.6	0.61	0.65	0.7	0.8
Strength of "Umami"	±	±	±	±	±
Strength of Flavor Enhancement	±	++	+++	+++++	+++++

Example 3

Formulation of Flavor Enhancement Seasoning I

MSG	1.5 parts by weight	
Potassium L-glutamate	1.5	"
NaCl	8	"
KCl	3	"
K ₂ HPO ₄	10	"
KH ₂ PO ₄	25	"
Sodium lactate	20	"
L-Histidine hydrochloride	20	"
DL-Alanine	4	"
L-Lysine hydrochloride	4	"
GSH	5	"

Flavor Enhancement Seasoning I was prepared by mixing the respective components according to the above formulation. A sample was then prepared by adding the seasoning I to beef consomme soup prepared in a conventional manner at a concentration of 1 g/dl. Using as a control beef consomme soup to which GSH had not been added, the beef consomme soup was organoleptically evaluated by a paired comparison test using a 20 member panel of judges. The results given in Table 15, show that the product of this invention enhanced the flavor such as thickness, amplitude, continuity etc. of the beef consomme soup. The beef consomme soup containing seasoning I was significantly more flavored as compared with the control.

Table 15

N = 20

	<u>Control</u>	<u>Present Invention</u>
Strength of Odor & Flavor	9	11
Strength of "Umami"	6	14**
Strength of Flavor Enhancement	2	18***
Total Preference	2	18***

*** Significantly different at a risk factor of 0.1%

** Significantly different at a risk factor of 1%

Example 4

Table 16
Formulations of Flavor
Enhancement Seasonings J - M
(parts by weight)

	<u>J</u>	<u>K</u>	<u>Control 1</u>	<u>L</u>	<u>M</u>	<u>Control 2</u>
Yeast Extract	100	100	100	100	100	100
MSG	-	-	-	1.5	1.5	1.5
IMP	-	-	-	1.5	1.5	1.5
GSH	1	20	-	1	20	-

Flavor Enhancement Seasonings J to M were prepared according to the formulations of Table 16. They were then organoleptically evaluated using 0.1% aqueous solutions of the respective samples by a paired comparison test method. The results are given in Table 17.

Table 17

	N = 10			
	<u>Paired Comparison</u> <u>against Control 1</u>		<u>Paired Comparison</u> <u>against Control 2</u>	
	J	K	L	M
Strength of Flavor Enhancement	+	++++	++++	+++++

As shown in Table 17, it has been confirmed that in yeast extract, GSH had an especially strong effect to impart flavor enhancement in the presence of appropriate amounts of MSG and IMP.

Example 5

30 Parts by weight of Flavor Enhancement Seasonings F and H and the control of Example 2 were added respectively to a seasoning liquor (pH 4.2, 1 g/dl) comprising 0.5 part by weight of citric acid, 0.6 part by weight of malic acid, 0.6 part by weight of fumaric acid, 0.15 part by weight of succinic acid, 0.5 part by weight sodium polyphosphate, 2.0 parts by weight of sorbitol and 0.4 part by weight of potassium sorbate.

500 g portions of preliminarily pickled radish were pickled in 300 ml portions of the respective seasoning liquors obtained above to prepare 3 kinds of radishes pickled in a jar. These were then organoleptically evaluated by a paired comparison test. The results are shown in Table 18. The radishes to which Flavor Enhancement Seasonings F and H had been added were superior to the control radishes in both flavor and preference.

Table 18

N = 20

	<u>Control</u> <u>Section</u>	<u>F Added</u> <u>Section</u>	<u>H Added</u> <u>Section</u>
Strength of Odor & Flavor	±	±	±
Strength of "Umami"	±	±	±
Strength of Flavor Enhancement	±	+++	++++
Preference	±	++++	++++

Example 6

Curry was prepared in a conventional manner using the recipe shown below and organoleptically evaluated for strength of flavor enhancement and total preference. The results shown in Table 19 demonstrate that the curry to which flavor enhancing Seasoning M had been added had stronger flavor and was significantly favored as compared with the control.

Recipe for Curry

Lard	8	Parts by weight
Low gluten flour	6	"
Curry powder	1.5	"
Table salt	1.7	"
Lecithin	0.04	"
Onion powder	0.3	"
Sugar	1.5	"
Flavor Enhancing Seasoning in Example 4	0.5	"
(or Control 2 in Example 4)	(0.5	")

Table 19

	N = 20	
	<u>Control 2</u> <u>Added Section</u>	<u>M Added</u> <u>Section</u>
Strength of Flavor Enhancement	4	16**
Total Preference	5	15*

** Significantly different at a risk factor of 1%

* Significantly different at a risk factor of 5%

Example 7

Pork sausages were prepared by using 650 parts by weight of pork, 50 parts by weight of lard, 50 parts by weight of corn starch, 5 parts by weight of spices, 25 parts by weight of a salting agent, 220 parts by weight of cold water and 5 parts of each of flavor enhancement Seasonings N, P, O. Pork sausages without seasoning were used as controls. The seasoning containing pork sausages and the control pork sausages were prepared by emulsification according to the formulation shown in Table 20. The pork sausages were prepared in a conventional manner, and organoleptically tested to find as shown in Table 21 that the pork sausages to which flavor enhancement Seasonings N, P and O had been added were significantly flavored as compared with that to which the control had been added.

Table 20
Formulations of
Mouthfulness Seasonings N, P & O

	<u>Parts By Weight</u>			
	<u>Control</u>	<u>N</u>	<u>P</u>	<u>O</u>
Pork extract	20	20	20	20
Pork fat	20	20	20	20
MSG	10	10	10	10
IMP	3	3	3	3
"Ajieki" (produced by Ajinomoto Co.)	30	30	30	30
Sugar ester	2	2	2	2
Reducing dextrin	15	15	15	15
GSH	-	2	10	20

Table 21

N = 10

	<u>Control Added Section</u>	<u>N Added Section</u>	<u>O Added Section</u>	<u>P Added Section</u>
Meaty taste	+	+	++	+++
Strength of Flavor Enhancement	±	+	+++	++++
Preference	+	++	++++	+++

Example 8

Tests confirmed that tomato juice, Japanese sake, commercial consomme soup, soy sauce, soybean taste soup, Worcester sauce and seaweed boiled down in soy sauce, either in which natural MSG and/or nucleic acid type taste intensifying substances had been already present or to which they were added at the time of production can be enhanced through imparting flavor enhancement by adding varied amounts of GSH to each. It was found that flavor enhancement was manifested in a variety of foods by adding an amount of GSH falling within the ranges shown in Table 22, to each.

Table 22

	<u>Effective GSH Addition Range</u>
Tomato juice	0.02 - 0.4 %
Japanese sake	0.001 - 0.1
Consomme soup	0.05 - 0.4
Soy sauce	0.05 - 0.4
Soybean paste soup	0.02 - 0.2
Worcester sauce	0.05 - 0.4
Seaweed boiled down in soy sauce	0.1 - 0.6

Example 9Table 23Flavor Enhancement Seasoning

	<u>A'</u>	<u>B'</u>	<u>C'</u>	<u>D'</u>	<u>E'</u>
MSG	80	75	50	80	-
IMP	-	5	-	3	30
GMP	-	-	10	3	30
GSO ₃ H	20	20	40	14	40

Flavor Enhancement Seasonings A' to E' were prepared by mixing the respective components according to the formulations outlined in Table 23. Using as a control in each instance a composition to which GSO₃H had not been added, aqueous solutions of 0.4 g/dl of the respective samples were prepared, and organoleptically evaluated by a paired comparison test. The results are given in Table 24.

Table 24

	<u>A'</u>	<u>B'</u>	<u>C'</u>	<u>D'</u>	<u>E'</u>
Strength of "Umami"	+	++	+++	+++	±
Strength of Flavor Enhancement	++	+++	++++	+++	+++

Example 10

Table 25
Formulations of Flavor
Enhancement Seasonings F' - K'

	(parts by weight)					
	<u>F'</u>	<u>G'</u>	<u>H'</u>	<u>I'</u>	<u>J'</u>	<u>K'</u>
HVP	60	60	60	60	-	-
HAP	-	-	-	-	60	60
MSG	26	26	26	26	26	26
IMP	1	1	1	1	1	1
GMP	1	1	1	1	1	1
2Na Succinate	2	2	2	2	2	2
NaCl	10	10	10	10	10	10
GSO ₃ H	1.7	8.3	16.7	33.3	1.7	33.3

Flavor Enhancement Seasonings F' to K' were prepared by the mixing respective components according to the above formulations. Using as each control a composition to which GSO₃H had not been added, organoleptic evaluation was conducted by a paired comparison test. The results are given in Table 26.

Table 26

	<u>Control</u>	<u>F'</u>	<u>G'</u>	<u>H'</u>	<u>I'</u>	<u>J'</u>	<u>K'</u>
Concentration (g/dl)	0.6	0.61	0.65	0.7	0.8	0.61	0.8
Strength of "Umami"	±	±	±	±	±	±	±
Strength of Flavor Enhancement	±	+	++	+++	+++	+	+++

Example 11

Consomme soups were prepared according to the following formulations and organoleptically evaluated for the strength of flavor enhancement and total preference. The evaluation shows that the consomme soup to which Flavor Enhancement Seasoning H' had been added had a stronger flavor and was significantly favored as compared with the control.

Recipe for Consomme Soup

Consomme soup stock (10 g of shavings of dried bonito)	1000 ml
Soy Sauce	3 ml
Table salt	6.5 g
Flavor Enhancement Seasoning of Example 10 (or Control Section in Example 10)	0.6 g

Table 27

	<u>Control Added Section</u>	<u>Flavor Enhancement Seasoning H' Added Section</u>
Strength of Flavor Enhancement	4	16 **
Preference of Flavor Enhancement	5	5 *

** Significantly different at a risk factor of 1%

* Significantly different at a risk factor of 5%

Example 12

Noodle soup was prepared using soy sauce, sweet sake, sugar, MSG, nucleic acid type seasoning, hydrolyzed vegetable protein and dried bonito extract according to the flavor recipe shown below. Organoleptic evaluations of the strength of the flavor and the taste of the noodle soup were conducted using a portions of the soup to which GSO_3H had not been added as a control. The evaluation was done by a paired comparison test, to find as shown in Table 28 that the portion of soup to which GSO_3H had been added had a strong flavor and also was significantly favored for total taste.

Recipe for Noodle Soup
(for use when eating)

Soy sauce	250 g
"Sake" (Japanese rice wine)	50
Sugar	50
"Ajinomoto"	5
IN	0.2
Hydrolyzed vegetable protein	5
Dried bonito extract	
(40g of flake of dried bonito was extracted with 700g of hot water)	700
GSO_3H	2

Table 28

	<u>Control Added Section</u>	<u>GSO₃H Added Section</u>
Strength of Flavor Enhancement	4	16**
Preference	4	16**

Example 13Table 29

	Flavor Enhancing Seasoning				
	<u>A"</u>	<u>B"</u>	<u>C"</u>	<u>D"</u>	<u>E"</u>
MSG	80	75	50	80	-
IMP	-	5	-	3	30
GMP	-	-	10	3	30
Lentinic acid	20	20	40	14	40

Flavor Enhancing Seasonings A" to E" were prepared by mixing the respective components according to the above formulations. Using as a control in each instance a portion to which lentinic acid had not been added, aqueous solutions of 0.4 g/dl of the respective samples were prepared and organoleptically evaluated by a paired comparison test. The results are given in Table 30.

Table 30

	<u>A"</u>	<u>B"</u>	<u>C"</u>	<u>D"</u>	<u>E"</u>
Strength of "Umami"	+	++	++++	+++	±
Strength of Flavor Enhancement	++	+++	++++	+++	+++

Example 14

Table 31

Formulations of Flavor
Enhancing Seasonings F"-K"

(parts by weight)

	<u>F"</u>	<u>G"</u>	<u>H"</u>	<u>I"</u>	<u>J"</u>	<u>K"</u>
HVP	60	60	60	60	-	-
HAP	-	-	-	-	60	60
MSG	26	26	26	26	26	26
IMP	1	1	1	1	1	1
GMP	1	1	1	1	1	1
2Na Succinate	2	2	2	2	2	2
NaCl	10	10	10	10	10	10
Lentinic Acid	1.7	8.3	16.7	33.3	1.7	33.3

Flavor Enhancing Seasonings F" to K" were prepared by mixing the respective components according to the above formulations. Using as a control in each case a formulation to which lentinic acid had not been added organoleptic evaluation was conducted by a paired comparison test similarly as in Example 13. The results are given in Table 32.

Table 32

	<u>Control</u>	<u>F"</u>	<u>G"</u>	<u>H"</u>	<u>I"</u>	<u>J"</u>	<u>K"</u>
Concentra- tion (g/dl)	0.6	0.61	0.65	0.7	0.8	0.61	0.8
Strength of "Umami"	±	±	±	±	±	±	±
Strength of Flavor Enhancement	+	+	++	+++	+++	+	+++

Example 15

Consomme soups were prepared according to the formulations given in Table 33 and organoleptically evaluated for strength of flavor and total preference. The evaluations given in Table 34 show that the consomme soup to which Flavor Enhancing Seasoning H" had been added had stronger flavor and was significantly favored as compared with the control.

Table 33Recipe for Consomme Soup

Consomme soup stock (10 g of flake of dried bonito)	1000 ml
Soy Sauce	3 ml
Table salt	6.5 g
Flavor Enhancing Seasoning of Example 14 (or Control Section in Example 14	0.6 g

Table 34

N = 20

	<u>Control Added Section</u>	<u>Flavor Enhancing Seasoning H" Added Section</u>
Strength of Flavor Enhancement	4	16 **
Preference	5	15 *
** Significantly different at a risk factor of 1%.		
* Significantly different at a risk factor of 5%.		

Example 16

Kombu Tsukudani (tangle boiled down in soy sauce) was prepared using tangle, soy sauce, sugar, MSG, nucleic acid type seasoning, HVP and starch syrup according to the following recipe given in Table 35. Organoleptic evaluations on the strength of flavor enhancement and the preference were conducted using a Kombu Tsukudani to which lentinic acid had not been added as a control. The evaluations were done by a paired comparison test, to find as shown in Table 35, that the lentinic acid added section had strong flavor enhancement and also was significantly favored for the preference.

Table 35
Recipe for Kombu Tsukudani

Tangle (after reconstitution in water)	1500 g
Soy sauce	1100 ml
Sugar	450 g
"Ajinomoto"	60 g
"WP"	2 g
"Ajimate"	30 g
Starch syrup	400 g
Lentinic acid	6 g

Table 36

	<u>Control Section</u>	<u>Lentinic Acid Added Section</u>
Strength of Flavor Enhancement	4	16 **
Preference	4	16 **

Example 17

	Flavor Enhancing Seasoning A "" (pts. by wt)	Flavor Enhancing Seasoning B "" (pts. by wt)	Flavor Enhancing Seasoning C "" (pts. by wt)	Flavor Enhancing Seasoning D "" (pts. by wt)	Flavor Enhancing Seasoning D _I "" (pts. by wt)
MSG	80	75	50	80	—
IMP	—	5	—	3	30
GMP	—	—	10	2	30
MMS	20	20	40	14	40
Total	100	100	100	100	100

Flavor Enhancing Seasonings A^{'''} to D_I^{'''} were prepared by mixing the respective components outlined in the above formulations. Using as a control a product to which MMS had not been added, aqueous solutions of 0.4 g/dl of the respective samples and solutions incorporating 0.1% by weight of the same respective samples in commercial tomato juice were prepared. Their organoleptic evaluation was conducted using a paired comparison test.

The results are given in Table 37 and Table 38.

Table 37
Evaluation of Simple Aqueous Solutions

	N = 20				
	<u>A^{'''}</u>	<u>B^{'''}</u>	<u>C^{'''}</u>	<u>D^{'''}</u>	<u>D_I^{'''}</u>
Strength of "Umami"	+	++	++++	+++	±
Strength of Characteristic Sweetness	+	+	+++	++	++
Strength of Flavor Enhancement	+++	++++++	++++++	++++++	+++++

Table 38

Evaluation of Addition to Commercial Tomato Juice
(each numeral indicates the number of persons
out of 20 who found the tomato juice containing
the Flavor Enhancing Seasoning preferable or better)

	<u>A "</u>	<u>B "</u>	<u>C "</u>	<u>D "</u>	<u>D_T "</u>
Strength of "Umami"	13	15*	18***	16**	15*
Strength of Characteristic Sweetness	14	15*	17**	14	17**
Strength of Flavor Enhancement	15*	17**	18***	18***	18***
Preference	15*	18***	18***	18***	18***
Remarks			Acid taste weakened	Acid taste weakened	Same as the left
			Strong tomato-like taste	Strong tomato-like taste	

Example 18

Table 39
Flavor Enhancing Seasonings

	<u>E'''</u>	<u>F'''</u>	<u>G'''</u>	<u>H'''</u>	<u>I'''</u>	<u>J'''</u>	<u>K'''</u>	<u>L'''</u>	<u>M'''</u>	<u>N'''</u>	<u>O'''</u>
HVP	60	60	60	60	--	--	--	--	30	15	15
HAP	--	--	--	--	--	--	60	60	15	15	30
YE	--	--	--	--	60	60	--	--	15	30	15
MSG	26	26	26	26	26	26	26	26	26	26	26
IMP	1	1	1	1	0	0	1	1	2	2	2
GMP	1	1	1	1	2	2	1	1	0	0	0
2Na Tartrate	2	2	2	2	--	--	2	2	2	2	2
NaCl	10	10	10	10	--	--	10	10	10	10	10
MMS	1.7	8.3	16.7	33.3	1.5	30	1.7	33.3	16.7	16.7	16.7

All by parts by weight.

0181421

Table 40

N = 10

	<u>E'''</u>	<u>F'''</u>	<u>G'''</u>	<u>H'''</u>	<u>I'''</u>	<u>J'''</u>	<u>K'''</u>	<u>L'''</u>	<u>M'''</u>	<u>N'''</u>	<u>O'''</u>
"Umami" Strength	±	±	±	±	±	±	±	±	±	±	±
Characteristic Sweetness	+	+	++	+++	+	+++	++	++++	+++	+++	+++
Flavor Enhancement	++	+++	++++	+++++	+++	++++	+++	++++	++++	++++	++++

Flavor Enhancement Seasonings E" to O" were prepared according to the formulations given in Table 39. (The tastiness strength, y value was adjusted to 3.8). Using each aqueous solution, organoleptic evaluations were conducted similarly to Example 17 by a paired comparison test using a control to which MMS had not been added.

The results are given in Table 40.

Example 19

Formulations consisting of 10 parts by weight of "Ajieki" (produced by Ajinomoto Co. Inc.), 0.1 part by weight of sodium succinate, 0.5 part by weight of 50% lactic acid, 0.5 part by weight of table salt and 1 part by weight of sugar were dissolved in 100 ml of water respectively, and 0.5 part by weight of Flavor Enhancing Seasonings G and O obtained in Example 2 were prepared. Controls were similarly prepared omitting the Flavor Enhancing Seasonings.

500 g of green asparagus was added to 500 ml of each of the seasoned solutions obtained by adding Flavor Enhancing Seasoning G and its control respectively. After boiling in a conventional manner, the green asparagus were subjected to organoleptic evaluation by a paired comparison test. Further, an

equal amount of corn was added to 500 ml of each of the seasoned solutions obtained by similarly adding Flavor Enhancing Seasoning O and its control respectively. After boiling in the conventional manner, these sample solutions were subjected to organoleptic evaluation by a paired comparison test. The results are as given in Table 41, in which the Flavor Enhanced products (those products to which Flavor Enhancing Seasonings G and O were added) were superior to the respective controls in the impartation of flavor, characteristic sweetness and in preference.

Table 41

N = 20

	<u>Green Asparagus</u>		<u>Sweet Corn</u>	
	<u>Control</u> <u>Section</u>	<u>G Added</u> <u>Section</u>	<u>Control</u> <u>Section</u>	<u>O Added</u> <u>Section</u>
Strength of Tastiness	12	18	11	9
Strength of Characteristic Sweetness	5	15 *	4	16 **
Strength of Flavor Enhancement	4	16 **	3	17 **
Preference	4	16 **	4	16 **

* Significantly different at a risk factor of 5%.

** Significantly different at a risk factor of 1%.

Example 20

Corn cream soup was prepared according to the formulation outlined below. Then 80 g of this formulation was fixed with 500 ml of cold water and, after boiling, organoleptically evaluated for the strength of flavor, vegetable-like taste and preference by a paired comparison test against a control formulation which had no added MMS. These evaluations reveal that, as shown in Table 42, the formulation with added MMS had a stronger flavor and was significantly favored for vegetable-like tastiness and total taste.

Formulation for Corn Cream Soup

Edible oil	27	Parts by weight
Flour	18	"
Dried onion flakes	12	"
Skimmed milk	12	"
Corn powder	15	"
Table salt	10	"
Onion powder	4	"
MSG	1	"
IMP	0.5	"
White pepper	0.5	"
MMS	1.0	"

Table 42

	N = 20	
	<u>Control</u> <u>Section</u>	<u>MMS Added</u> <u>Section</u>
Strength of Flavor Enhancement	4	16 *
Vegetable-like Tastiness	4	16 *
Preference	4	16 *

* Significantly different at a risk factor of 5%.

Example 21

A sea-urchin egg "Nerium" paste was prepared according to the formulation outlined below. This paste was organoleptically evaluated for strength of flavor, characteristic sweetness and total preference by a pair comparison test method. This evaluation, shown in Table 43 demonstrates that the formulations having added MMS flavor and was significantly favored as compared with the control (MMS not added).

Formulation for Sea Urchin Egg Paste

Raw material sea urchin egg	5	Parts by weight
Powdered egg	1	"
Alcohol	0.8	"
MSG	0.4	"
Glycine	0.4	"
Methionine	0.1	"
β -Carotin	q.s.	"
Water	22.3	"
MMS	0.3	"

Table 43

N = 20

	<u>Control Section</u>	<u>MMS Added Section</u>
Strength of Sweetness	6	14
Strength of Flavor Enhancement	3	17 **
Preference	5	15 **

* Significantly different at a risk factor of 5%.

** Significantly different at a risk factor of 1%.

Example 22

A seaweed composition for rice to be eaten with hot green tea "Uchazuka" was prepared according to the formulation shown below. 8 g of the prepared composition was placed on top of boiled rice and hot green tea was poured to prepare rice and seaweed doused in hot green tea. The composition was then organoleptically evaluated for strength of flavor and total preference by a pair comparison test method using a control to which no MMS had been added. These evaluations as shown in Table 44, the MMS added section had stronger flavor and was significantly favored.

Formulation for Seaweed Composition
For Rice with Hot Green Tea

Tangle powder	0.5	Parts by weight
Powdered tea	2.0	"
Sugar	3.0	"
Table salt	53.0	"
"Ajinomoto"	10.0	"
Glycine	3.0	"
Shredded seaweed	5.0	"
Rice cake pellets	5.0	"
MMS	0.5	"

Table 44

N = 20

	<u>Control Section</u>	<u>MMS Added Section</u>
Strength of Flavor Enhancement	4	16 **
Preference	5	15 *

Example 23Table 45Flavor Enhancing Seasoning

	<u>A""</u>	<u>B""</u>	<u>C""</u>	<u>D""</u>	<u>E""</u>
MSG	80	75	50	80	--
IMP	--	5	--	3	30
GMP	--	--	10	3	30
Cycloalliin	20	20	40	14	40

The respective components outlined above were mixed to prepare Flavor Enhancing Seasonings A"" to E"". A product to which cycloalliin had not been added was used as a control in each case. Aqueous solutions containing 0.4 g/dl of the respective samples were prepared, and organoleptical evaluation was conducted by a pair comparison test method. The results are given in Table 46.

Table 46

	<u>A"</u>	<u>B"</u>	<u>C"</u>	<u>D"</u>	<u>E"</u>
Strength of "Umami"	+	++	++++	+++	±
Strength of Flavor Enhancement	++	+++	++++	+++	+++

Example 24

Table 47

Formulations of Flavor
Enhancing Seasonings F" - K "

	(parts by weight)					
	<u>F"</u>	<u>G"</u>	<u>H"</u>	<u>I"</u>	<u>J"</u>	<u>K"</u>
HVP	60	60	60	60	--	--
HAP	--	--	--	--	60	60
MSG	26	26	26	26	26	26
IMP	1	1	1	1	1	1
GMP	1	1	1	1	1	1
2Na Succinate	2	2	2	2	2	2
NaCl	10	10	10	10	10	10
Cycloalliin	1.7	8.7	16.7	33.3	1.7	33.3

The respective components outlined above were mixed to prepare Flavor Enhancing Seasonings F" to K". Using, as each control a product from which the cycloalliin had been omitted, organoleptic evaluations

were conducted similarly as outlined in Example 23 by a pair comparison test method. The results are given in Table 48.

<u>Table 48</u>							
	<u>Control</u>	<u>F""</u>	<u>G""</u>	<u>H""</u>	<u>I""</u>	<u>J""</u>	<u>K""</u>
Concentration (g/dl)	0.6	0.61	0.65	0.7	0.8	0.61	0.8
Strength of "Umami"	±	±	±	±	±	±	±
Strength of Flavor Enhancement	±	+	++	+++	+++	+	+++

Example 25

Consomme soups were prepared according to formulations outlined below. These were organoleptically evaluated for the strength of flavor and total preference, to find that the consomme soup to which Flavor Enhancing Seasoning H"" had been added had stronger flavor and was significantly favored as compared with the control.

Recipe for Consomme Soup

Consomme soup stock (10 g of shavings of dried bonito)	1000 ml
Soy Sauce	3 ml
Table salt	6.5 g
Flavor Enhancing Seasoning of Example 24 (or Control Section in Example 24)	0.6 g

Table 49

	<u>Control Added Section</u>	<u>Flavor Enhancing Seasoning H" Added Section</u>
Strength of Flavor	4	16 **
Preference of Flavor	5	15 *

** Significantly different at a risk factor of 1%.

* Significantly different at a risk factor of 5%.

Example 26

Chinese noodle soup was prepared using the formulation outlined below. The soup was then organoleptically evaluated for the strength of flavor and total preference by a pair comparison test method. This evaluation shows that the Chinese noodle soup to which Flavor Enhancing Seasoning H"" had been added had stronger flavor and was significantly favored as compared with the control.

Formulation of Chinese Noodle Soup

Soy sauce	40	Parts by weight
Table salt	16	"
Lard	24	"
Sesame Oil	2	"
"Ajimate"	2	"
YE	1	"
Vegetable extract	8	"
Flavor Enhancing Seasoning in Example 24 (or Control Section 7 in Example 24)	7	"

Table 50

N = 20

	<u>Control Section</u>	<u>Flavor Enhancing Seasoning H" Added Section</u>
Strength of Flavor	4	16 **
Total Preference	5	15 *

** Significantly different at a risk factor of 1%.

* Significantly different at a risk factor of 5%.

Experimental Example 9

(Evaluation in Simple Aqueous Solution Systems)

Using as a control a mixed aqueous solution of 0.05 g/dl of MSG and 0.05 g/dl of IMP (y value = 3.09), sample solutions were prepared by adding 0.002 to 0.5 g/dl of alliin to portions of the mixed aqueous solution. These sample solutions were then organoleptically evaluated by paired comparison test against the control using an organoleptic panel of 20 members (N = 20). The results are given in Table 51.

Table 51

	<u>Formulation</u>			<u>Taste Intensifying Effect</u>	
	<u>MSG g/dl</u>	<u>IMP g/dl</u>	<u>Alliin g/dl</u>	<u>Strength of "Umami"</u>	<u>Strength of Flavor Enhancement</u>
Control	0.05	0.05	--	±	±
Sample AA	"	"	0.002	±	±
BB	"	"	0.02	±	+
CC	"	"	0.05	±	++
DD	"	"	0.1	+	+++
EE	"	"	0.3	±	++++
FF	"	"	0.5	±	+++++
GG	--	0.1	0.1	-	+++

As shown in Table 50, with the amount of added alliin less than 0.1% based on the y value (MSG calculated amount), the strength of flavor is comparable to that of the control. But by the addition of 0.1% or more, the flavor alone is remarkably increased without changing the strength of "Umami". If the absolute concentration of alliin exceeds 1.0%, a specific thickness in taste is manifested and the total taste balance is lost. Therefore, the amount of alliin added should suitably be in the range of 0.1 to 1000% by weight, preferably 0.1 to 500% by weight, based on the weight of co-present component calculated as the weight of MSG having the same "Umami" strength. Further, alliin manifests the most preferred effect in the presence of an appropriate amount of the above "Umami" component, that is, when the above y value is 0.1 to 30 as the concentration at the time of eating.

When a nucleic acid type taste intensifying substance is used alone, if alliin is added at a ratio of less than 1/99 to the nucleic acid type taste intensifying substance, the flavor enhancing effect is not adequate. Whereas if this ratio exceeds 99/1, specific thickness in taste is brought about and the total taste balance is lost.

Experimental Example 10

(Evaluation in Garlic Extract Systems)

The effect of the addition of alliin to a formulation of major components of garlic extract was evaluated by paired comparison test against a control which contained no alliin. The results are given in Table 53, from which it has been confirmed that alliin imparts a higher natural taste by the manifestation of strong flavor with synthetic garlic extract.

Table 52
Formulation of Major
Components of Garlic Extract

	<u>Formulation</u>
Asp	3.6 %
Glu	3.3
Lys	6.1
Arg	42.3
Other amino acids *	18.7
NaCl	19.7
KCl	6.7
Citric acid	0.1
Succinic acid	<u>0.1</u>
	100

* Ser, Val and Pro

Table 53

	<u>Major Components g/dl</u>	<u>Alliin g/dl</u>	<u>Strength of Flavor</u>	<u>Strength of Natural Taste</u>
Control	1.0	0	±	-
Sample AA	"	0.03	+	±
BB	"	0.1	++	+
CC	"	0.3	+++	++

Alliin in this invention may be that obtained by any production process, such as an extraction process, a synthesis process etc. Further, it is also possible to replace the whole or part of the required alliin by a substance containing alliin at a relatively high concentration, for example, garlic extract, onion extract or other alliin extract.

Experimental Example 11

(Comparison of Taste Intensifying Powers of Representative Compounds)

Using as a control a mixed aqueous solution of 0.05 g/dl of MSG and 0.05 g/dl of IMP (γ value = 3.09), sample solutions were prepared by adding 0.1 g/dl of the respective sample compounds shown in Table 52 to the mixed solutions. These sample solutions were evaluated by a paired comparison test by an organoleptic panel consisting of 20 members (N = 20). The results are shown in Table 54.

Table 54

(N = 20)

	<u>"Umami"</u>	<u>Flavor</u>
(1) Control	±	±
(2) S-Methylcysteinesulfoxide	±	+++
(3) S-Ethylcysteinesulfoxide	±	++
(4) S-Propylcysteinesulfoxide	±	++
(5) S-Propenylcysteinesulfoxide	±	+++
(6) γ-Glutamylcysteine	±	+
(7) γ-Glutamyl-S-methylcysteine	±	+
(8) γ-Glutamyl-S-ethylcysteine	±	+
(9) γ-Glutamyl-S-propylcysteine	±	+
(10) γ-Glutamyl-S-propenylcysteine	±	+
(11) γ-Glutamyl-S-allylcysteine	±	++
(12) γ-Glutamyl-S-methylcysteinesulfoxide	±	++
(13) γ-Glutamyl-S-ethylcysteinesulfoxide	±	++
(14) γ-Glutamyl-S-propylcysteinesulfoxide	±	+++
(15) γ-Glutamyl-S-propenylcysteinesulfoxide	±	+++
(16) γ-Glutamyl-S-allylcysteinesulfoxide	±	+++
(17) S-(2-Carboxypropyl)glutathione	±	++
(18) S-(3-Carboxy-3-methylethyl)glutathione	±	++

Experimental Example 12

Using the same mixed aqueous solution of MSG and IMP as that used in Experimental Example 11 as a control, samples were prepared by adding thereto 0.001

to 1.0 g/dl of the sample compounds outlined in Table 54. Aqueous solutions of 0.1 g/dl of IMP and 0.1 g/dl of the respective sample compounds outlined in Table 54 were also prepared as samples. All of these were evaluated by a pair comparison test method against a control by an organoleptic panel consisting of 20 members (N = 20). Some of the results are given in Table 55 (the case of γ -glutamyl-S-allylcysteinesulfoxide).

Table 55

	<u>Formulation</u>			<u>Taste Intensifying Effect</u>	
	<u>MSG g/dl</u>	<u>IMP g/dl</u>	<u>Compound No. 16 in Experimental Example 11 g/dl</u>	<u>Strength of "Umami"</u>	<u>Strength of Flavor</u>
Control	0.05	0.05	—	±	±
Sample AA	"	"	0.002	±	±
BB	"	"	0.01	±	+
CC	"	"	0.05	±	++
DD	"	"	0.10	+	+++
EE	"	"	0.50	±	+++
FF	"	"	1.00	±	+++
GG	—	0.1	0.10	-	++

As shown in Table 55, with an added amount of less than 0.1% based on the y value (MSG calculated amount), the strength of flavor is unchanged. However, with the addition of 0.1% or more, the flavor is remarkably increased. In this case, if the absolute concentration of this compound (compound No. 16 of Experimental Example 11) exceeds 1.0%, specific thickness in taste is manifested and the total taste balance is lost. Therefore, the amount of this compound added should suitably be in the range of 0.1 to 1000% by weight, preferably 0.1 to 50% by weight, based on the co-present "Umami" component calculated as the weight of MSG having the same "Umami" strength. Further, the compound manifests the most preferred effect in the presence of an appropriate amount of the above "Umami" component, that is, when the above y value is 0.1 to 30 as the concentration at the time of eating. The above fact likewise applies to the other compounds of the general formulae (1) and (2).

On the other hand, in the case where the nucleic acid type taste intensifying substance is used alone, if compound (1) or (2) is added in an amount of less than 1/99 at the ratio to the nucleic acid type taste intensifying substance, the flavor enhancing effect is not adequate. Whereas if this ratio exceeds 99/1, a

specific thickness in taste is brought about and the total taste balance is lost. This also applies to the other compounds of the general formulae (1) and (2).

Experimental Example 13

(Evaluation in Garlic Extract Systems)

Each compound set forth in Table 54 was added to a formulation comprising major components of garlic extract (set forth in Table 55). The compositions were evaluated by a paired comparison test using a control which contained no addition of compounds of Table 54. The results are given in Table 57 from which it has been confirmed that also in the formulated garlic extract, these compounds impart a higher natural flavor.

Table 56
Formulation of Major Components
in Garlic Extract

	<u>Formulation</u>
Asp	3.6 %
Glu	3.3
Lys	6.1
Arg	42.3
Other amino acids*	18.7
NaCl	19.4
KCl	6.7
Citric acid	0.1
Succinic acid	0.1

* Ser, Val, Pro

Table 57

<u>Compound Added</u>	<u>Concentration of Formulated Extract (g/dl)</u>	<u>Concentration of Compound Added (g/dl)</u>	<u>Strength of Flavor Enhancement</u>	<u>Strength of Natural Flavor</u>
(1) Control	1.0	0	±	-
(2) S-Methylcysteine-sulfoxide	"	0.1	++	+
(3) S-Ethylcysteine-sulfoxide	"	0.1	+	+
(4) S-Propylcysteine-sulfoxide	"	0.1	++	+
(5) γ-Glutamyl-cysteine	"	0.1	+	±
(6) γ-Glutamyl-S-allylcysteine	"	0.1	++	+
(7) γ-Glutamyl-S-methylcysteine	"	0.1	++	+
(8) γ-Glutamyl-S-methylcysteine	"	0.1	+	±
(9) γ-Glutamyl-S-propylcysteine	"	0.1	+	±
(10) γ-Glutamyl-S-propenyl-cysteinesulfoxide	"	0.1	++	+
(11) γ-Glutamyl-S-allylcysteine-sulfoxide	"	0.03	+	+
(12) γ-Glutamyl-S-allylcysteine sulfoxide	"	0.1	++	++
(13) γ-Glutamyl-S-allylcysteine-sulfoxide	"	0.3	+++	++

The compounds of the general formulae (1) and (2) may be used singly or as a mixture of two or more. They can also be wholly or partially replaced by substances which contain considerably high concentrations of these compounds, such as garlic extract (preferably, extract from which the odor components have been removed by deodorizing process e.g. the processed described in Japanese Patent Application No. 82169/1983), onion extract etc. Similarly, the "Umami" components such as glutamic acid, nucleic acid type tastiness substances etc. can also be wholly or partially replaced by "Umami" components contained in the food or seasoning per se to which these compounds are to be added.

Examples of the "Umami" component which may be used in this invention include, in addition to MSG, IMP, GMP etc., any component having a "Umami" imparting effect. Some of these are: potassium glutamate, calcium glutamate, potassium 5'-inosinate, calcium 5'-inosinate, potassium 5'-guanylate, calcium 5'-guanylate etc. and any food or seasoning which contains said "Umami" components as one of its constituting components; for example, protein hydrolysates (HAP, HVP), yeast extract, meat extract, vegetable extract etc.

The Flavor Enhancing seasonings of this invention may be prepared by any process, for example, by merely dry blending MSG, IMP, GMP etc. with these compounds by pelletizing them using an appropriate excipient, etc. Similarly, for the production of food having enhanced flavor according to this invention, the actual manner for the production of the food is not limited as long as the "Umami" components and these compounds are co-present in the final product at the above-described mixing ratio.

In addition, it is needless to say that the use in combination of seasonings, for example, salty taste agents such as table salt, potassium chloride etc., sweetening agents such as sugar etc., acid taste agents such as organic acids etc., flavors, spices, quality modifiers etc. may be possible unless it departs from the object of this invention.

This invention is more particularly described by the following examples.

Example 27Table 58Flavor Enhancing Seasoning

	<u>AA</u>	<u>BB</u>	<u>CC</u>	<u>DD</u>	<u>EE</u>
MSG	80	75	50	80	
IMP		5		3	30
GMP			10	3	30
S-Methylcysteine-sulfoxide	20	20	40	14	40

Of the compounds of the general formulae (1) and (2), the exemplary case of S-methylcysteinesulfoxide is set forth in Table 58. According to this formulation, the respective components were mixed to prepare Flavor Enhancing Seasonings AA to EE. As each control, a product to which S-methylcysteinesulfoxide had not been added was used. Aqueous solutions containing 0.4 g/dl of the respective sample compounds (outlined in Table 57) and solutions obtained by adding 0.1% by weight of the sample compounds to commercial consomme soup were prepared. An organoleptic evaluation was conducted by a paired comparison test. The results are given in Table 59 and Table 60.

Table 59

	<u>AA</u>	<u>BB</u>	<u>CC</u>	<u>DD</u>	<u>EE</u>
Strength of "Umami"	+	++	++++	+++	±
Strength of Flavor	++	+++	+++	+++	+++

Table 60

Evaluation When Added to
Commercial Consomme Soup

	<u>AA</u>	<u>BB</u>	<u>CC</u>	<u>DD</u>	<u>EE</u>
Strength of "Umami"	12	16 **	18 ***	17 **	15 *
Strength of Flavor	13	15 *	17 **	13	16 **
Taste	14	18 ***	18 ***	18 ***	18 ***

N = 20

Each numeral in the table indicates the number of persons who have found it preferable or strong.

- * Significantly different at a risk factor of 5%.
- ** Significantly different at a risk factor of 1%.
- *** Significantly different at a risk factor of 0.5%.

Example 28

Table 61
Flavor Enhancing Seasonings

	(parts by weight)					
	<u>FF</u>	<u>GG</u>	<u>HH</u>	<u>II</u>	<u>JJ</u>	<u>KK</u>
HVP	60	60	60	60	--	--
HAP	--	--	--	--	60	60
MSG	26	26	26	26	26	26
IMP	1	1	1	1	1	1
GMP	1	1	1	1	1	1
2Na Succinate	2	2	2	2	2	2
NaCl	10	10	10	10	10	10
Sample Compound *	1.7	8.3	16.7	33.3	1.7	33.3

* Mixture of equal amounts of S-methylcysteinesulfoxide, S-propenylcysteinesulfoxide, S-propylcysteinesulfoxide and γ -glutamyl-S-allylcysteine-sulfoxide.

According to the above formulation, the respective components were mixed to prepare Flavor Enhancing Seasonings FF to KK. Using as each control a product to which the sample compound had not been added, organoleptic evaluation was conducted as outlined in Example 27 by a paired comparison test. The results are given in Table 62.

Table 62

	<u>Control</u>	<u>FF</u>	<u>GG</u>	<u>HH</u>	<u>II</u>	<u>JJ</u>	<u>KK</u>
Concentration (g/dl)	0.6	0.61	0.65	0.7	0.8	0.61	0.8
Strength of "Umami"	±	±	±	±	±	±	±
Strength of Flavor	±	+	++	+++	+++	+	+++

Example 29

Chinese noodle soup was prepared using the formulation shown below, and organoleptically evaluated for strength of flavor and total preference by a paired comparison test. These evaluations show that the Chinese noodle soup to which Flavor Enhancing Seasoning HH had been added had stronger flavor and was significantly favored as compared with the control.

Formulation of Chinese Noodle Soup

Soy sauce	40	Parts by weight
Table salt	16	
Lard	24	
Sesame oil	2	
"Ajimate"	2	
YE	1	
Vegetable extract	8	
Flavor Enhancing Seasoning in Example 28 (or Control Section in Example 28)	7	

Table 63

N = 20

	<u>Control</u> <u>Section</u>	<u>Flavor Enhancing</u> <u>Seasoning HH</u> <u>Added Section</u>
Strength of Flavor	4	16 **
Total Preference	5	15 *

** Significantly different at a risk factor of 1%.

* Significantly different at a risk factor of 5%.

Example 30

Table 64

	<u>LL</u>	<u>MM</u>	<u>NN</u>	<u>OO</u>	<u>PP</u>
MSG	80	75	50	80	--
IMP	--	5	--	3	30
GMP	--	--	10	3	30
Alliin	20	20	40	14	40

The respective components outlined in the above formulations were mixed to prepare Flavor Enhancing Seasonings LL to PP. As each control, a product to which alliin had not been added was used. Aqueous solutions containing 0.4 g/dl of the respective formulations and solutions obtained by adding 0.1% by

weight of the above formulations to commercial consomme soup were prepared. Organoleptical evaluations were conducted by a paired comparison test. The results are given in Table 65 and Table 66.

Table 65

	<u>LL</u>	<u>MM</u>	<u>NN</u>	<u>OO</u>	<u>PP</u>
Strength of Tastiness	+	++	++++	+++	±
Strength of Flavor	+++	++++	+++++	++++	++++

Table 66

Evaluation When Added to
Commercial Consomme Soup

N = 20

(Each numeral indicates the number of persons out of 20 who have found it preferable or strong).

	<u>LL</u>	<u>MM</u>	<u>NN</u>	<u>OO</u>	<u>PP</u>
Strength of "Umami"	12	16 **	18 ***	17 **	15 **
Strength of Flavor	13	15 *	17 **	13	16 **
Taste	14	18 ***	18 ***	18 ***	18 ***
Comment	Aftertaste Aftertaste Strong, Strong, Flavor Flavor Strong & Strong & Preferred Preferred				

* Significantly different at a risk factor of 5%.

** Significantly different at a risk factor of 1%.

*** Significantly different at a risk factor of 0.5%.

Example 31

Table 67
Formulations of Flavor Enhancing
Seasonings QQ to VV

	(parts by weight)					
	<u>QQ</u>	<u>RR</u>	<u>SS</u>	<u>TT</u>	<u>UU</u>	<u>VV</u>
HVP	60	60	60	60	-	-
HAP	--	--	--	--	60	60
MSG	26	26	26	26	26	26
IMP	1	1	1	1	1	1
GMP	1	1	1	1	1	1
2Na Succinate	2	2	2	2	2	2
NaCl	10	10	10	10	10	10
Alliin	1.7	8.7	16.7	33.3	1.7	33.3

The respective components outlined in the above formulations were mixed to prepare Flavor Enhancing Seasonings QQ to VV. Using, as each control, a product from which the alliin had been omitted, organoleptic evaluation was conducted as outlined in Example 30 by a paired comparison test. The results are given in Table 68.

Table 68

	<u>Control</u>	<u>QQ</u>	<u>RR</u>	<u>SS</u>	<u>TT</u>	<u>UU</u>	<u>VV</u>
Concentration (g/dl)	0.6	0.61	0.65	0.7	0.8	0.61	0.8
Strength of Umami	±	±	±	±	±	±	±
Strength of Flavor	±	++	+++	++++	++++	++++	++++

Example 32

Using the following recipe, spaghetti meat sauce was prepared and strength of Flavor and total preference were organoleptically evaluated by paired comparison test. These evaluations, as shown in Table 68, demonstrate that meat sauce to which Flavor Enhancing Seasoning SS had been added had stronger flavor and was significantly favored as compared with the control.

Recipe for Meat Sauce

Minced beef and pork	15.7
Onion	39.2
Carrot	4.7
Corn salad oil	7.8
Flour	7.8
Tomato ketchup	15.7
Tomato puree	4.7
Worcestershire sauce	3.8
Flavor Enhancing seasoning of Example 31 (or Control Section in Example 31)	0.6

Table 69

N = 20

	<u>Control Added Section</u>	<u>Flavor Enhancing Seasoning SS Added Section</u>
Strength of Flavor	4	16 **
Total Preference	5	15 **

** Significantly different at a risk factor of 1%.

* Significantly different at a risk factor of 5%.

Example 33

Barbecue sauce was prepared from refined spices, soybean oil, soy sauce, soybean paste (miso), granulated sugar, MSG, starch, synthesized rice wine (sake) and guar gum according to the recipe outlined below. The strength of flavor and taste were organoleptically evaluated by a paired comparison test using a sample to which alliin had not been added as a control. As shown in Table 70 the alliin added section had strong flavor and was significantly favored for the total taste.

Recipe for Barbecue Sauce

Spices	12.5	Parts by weight
Refined soybean oil	25	"
Soy sauce	34	"
Soybean paste (miso)	9	"
Granulated sugar	8	"
MSG	1	"
Starch	1	"
Synthesized rice wine (sake)	9	"
Guar gum	0.1	"
Alliin	0.5	"

Table 70

	<u>Control Section</u>	<u>Alliin Added Section</u>
Strength of Flavor	4	16 **
Total Taste	4	16 **

Example 34

Hamburgers were prepared as outlined in Table 70 using 30 parts of pork, 15 parts of chicken, 5 parts of beef, 4 parts of bread crumb, 2 parts of "AJIPRON" (produced by Ajinomoto Co.), 4 parts of "SHLN-ESUSAN" starch (produced by Ajinomoto Co.), 17 parts of onion and 5 parts of each of Flavor Enhancing Seasonings WW, XX and YY. Controls were prepared according to the same formulations but without the Flavor Enhancing Seasonings. These hamburgers were organoleptically evaluated to find as shown in Table 72 that the hamburgers to which Flavor Enhancing Seasonings WW, XX and YY had been added respectively had stronger flavor and were significantly favored as compared with that to which the control had been added.

Table 71
Formulations of Flavor
Enhancing Seasonings W, X and Y

	<u>WW</u>	<u>XX</u>	<u>YY</u>
Table salt	8	8	8
Soy sauce	40	40	40
Sugar	8	8	8
(Ajinomoto Co.)	8	8	8
Wine	10	10	10
MSG	10	10	10
White pepper	6	6	6
Nutmeg	4	4	4
Ginger	6	6	6
Alliin	2	10	20

Table 72

	<u>Control</u> <u>Added</u> <u>Section</u>	<u>WW Added</u> <u>Section</u>	<u>XX Added</u> <u>Section</u>	<u>YY Added</u> <u>Section</u>
Strength of Flavor	±	+	+++	+++++
Preference	+	++	++++	+++

Example 35

Mix Vegetable Extracts WW', XX' and YY' prepared in a conventional manner using the formulations of Table 73. These were evaluated in Chinese noodle soup

to find as shown in Table 74 that the Chinese noodle soup to which the mix vegetable extracts had been added respectively had stronger flavor and each was significantly favored as compared with control which contained no mix vegetable extract.

Table 73
Formulations of Mix Vegetable
Extracts WW', XX' and YY'

	<u>Control</u>	<u>WW'</u>	<u>XX'</u>	<u>YY'</u>
Onion	60	60	60	60
Garlic	30	30	30	30
Leek	5	5	5	5
Carrot	5	5	5	5
Alliin	--	5	10	15

Recipe for Chinese Noodle Soup

Soy sauce	40	Parts by weight
Table salt	16	"
Lard	24	"
Sesame oil	2	"
WP	1	"
"Ajinomoto"	6	"
"Ajimate"	2	"
YE	1	"
Aforesaid Mix Vegetable Extract	8	"

Table 74

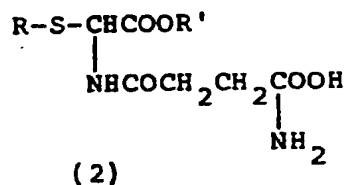
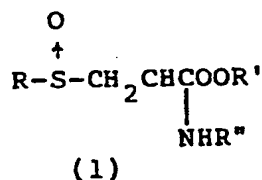
	<u>Control</u>	<u>WW'</u>	<u>XX'</u>	<u>YY'</u>
Strength of Flavor	±	+	++	++++
Preference	+	++	++++	++++

Obviously, numerous modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described herein.

CLAIMS

1. A composition comprising

- i) a taste intensifying substance, and
- ii) a flavor enhancing substance which is at least one member selected from glutathione, salts of glutathione, oxidized glutathione, salts of oxidized glutathione, glutathione sulfonic acid, salts of glutathione sulfonic acid, cycloallin, salts of cycloallin, lenitinic acid, salts of lentinic acid, des-glutamyl lentinic acid, salts of des-glutamyl lentinic acid, methionine methyl sulfonium, salts of methionine methyl sulfonium, compounds of the formulae



wherein R is a saturated or unsaturated aliphatic hydrocarbon group of from 1 to 3 carbon atoms or a carboxyalkyl group wherein said alkyl is from 1 to 6 carbon atoms,

R' is H, a carboxymethyl amino group or a salt of a carboxymethyl amino group, and

R" is H, a γ -glutamyl group or a salt of a γ -glutamyl group,

salts of compound (1) and salts compound (2).

2. The composition of Claim 1 wherein said taste intensifying substance comprises glutamic acid, a salt of glutamic acid or a mixture thereof.

3. The composition of Claim 2 wherein said flavor enhancing substance is used in an amount in the range of 0.1 to 1000% by weight based on the weight of said taste intensifying substance.

4. The composition of Claim 3 wherein said taste intensifying substance is used in an amount sufficient to obtain an adequate taste intensifying strength.

5. The composition of Claim 1 wherein said taste intensifying substance comprises a nucleic acid or a salt of a nucleic acid.

6. The composition of Claim 5 wherein said taste intensifying substance comprises 5'-inosinate, a salt of 5'-inosinate, 5'-guanylate, a salt of 5'-guanylate or a mixture thereof..

7. The composition of Claim 5 wherein said flavor enhancing substance is used in an amount in weight ratio of 1:99 to 99:1 relative to said taste intensifying substance.

8. The composition of Claim 1 wherein said taste intensifying substance comprises glutamic acid or a salt of glutamic acid, said taste intensifying substance further comprising at least one member selected from 5'-inosinate, a salt of 5'-inosinate, 5'-guanylate and a salt of 5'-guanylate;

wherein the taste intensifying strength of said substance is determined as follows:

i) for 5'-inosinate or a salt thereof:

$$y = u + 1218 uv$$

ii) for 5'-guanylate or a salt thereof:

$$y = u + 2800 uv;$$

wherein

y is the taste intensifying strength,

u is the glutamic acid or glutamic acid salt concentration expressed as a weight %,

v is the 5'-inosinate or 5'-inosinate salt concentration expressed as a weight %, or

v is the 5'-guanylate or 5'-guanylate salt concentration expressed as a weight %; and

y is in the range of 0.1 to 30 weight %.

9. A food which comprises the composition of any one of the preceding claims.

10. A food comprising the composition of any one of the claims 1 to 8 wherein said food comprises a pork sausage, tomato juice, Japanese sake, consomme soup, noodle soup, corn cream soup, soy sauce, soybean taste soup, Worcester sauce, seaweed boiled down in soy sauce, sea urchin egg paste, beef extract, shrimp extract, onion extract, curry, garlic extract or rice.

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14th October 1985

Our Ref.

Your Ref.

IS/NJH/A-605

Dear Sirs,

European Patent Application No. 84.307770.2
AJINOMOTO CO., LTD.

I am writing to put forward some amendments, as shown on the enclosed copies of pages 3,4,11,12,70 and 92. These arise from the discovery of errors in formulae (1) and (2) and their definitions. These occur in the description on pages 3,4,11 and 12; in claim 1; and in the abstract. There is also an error in Table 54.

That there are errors is obvious. The Examiner who revised the abstract appreciated that something was amiss, and tried to correct it by omitting a methylene group (-CH₂-) from formula (1). However, this makes matters worse.

Examples of compounds intended to be covered by formulae (1) and (2) appear in Table 54 on page 70. (That this is the intention is shown by various passages, e.g. on page 72, paragraph 1, last two lines.)

Formula (2) is a sulfide and was thus intended to include compounds 6-11,17 and 18. All of the compounds 2-18 in Table 54 can be regarded as derivatives of cysteine. Thus if R"=glutamyl, (1) should be the S-oxidised form of (2). As shown, however, they differ by a methylene group. The necessary correction is to insert this group into formula (2), otherwise it cannot represent any of the compounds disclosed.

Formulae (1) and (2) (as filed) include the group -COOR', and R' is defined as H, carboxymethylamino, or a salt thereof. (There is a further error in the abstract, where the definitions of R' and R" are telescoped, the typist having apparently jumped from the "H" of "R'" to the "H" of "R" is H".) This group -COOR' is reasonable when R'=H, making the group a carboxy

European Patent Office

14th October 1985

group. However, when $R' = \text{carboxymethylamino}$ or a salt thereof (i.e. $-\text{NH}.\text{CH}_2.\text{CO}_2\text{Z}$) this gives the manifestly incorrect result $-\text{CO}.\text{O}.\text{NH}.\text{CH}_2.\text{CO}_2\text{Z}$. It must be apparent that there is an "O" too many. Nitrogen should be connected to the carbonyl carbon atom directly, to give $-\text{CO}.\text{NH}.\text{CH}_2.\text{CO}_2\text{Z}$. This is confirmed by compounds 17 and 18. These are derivatives of glutathione, which can be related to formula (2) (after insertion of the missing methylene group) only if $-\text{COOR}'$ is corrected to $-\text{COR}'$. Of course, if the formulae are amended in this way, the condition $R' = \text{H}$ must be amended to $R' = \text{OH}$.

The definition of R has been amended to refer to "aliphatic hydrocarbon group of from 1 to 4 carbon atoms" (in place of "...from 1 to 3...").

There are minor errors in the name of compound 18 in Table 54. Clearly both occurrences of "3" are incorrect. The intended character was "ß"; but "2" may be preferred.

Compound 6 corresponds to (amended) formula (2) when $R = \text{H}$. This was originally excluded, but the definition of R has been amended to include it.

Yours faithfully,



I.M. Armitage
Authorised Representative

Encs: Pages 3,4,11,12,70 and 92



European Patent
Office

EUROPEAN SEARCH REPORT

0181421
Application number

EP 84 30 7770

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
A	US-A-3 214 276 (SAKAKIBARA et al.) * Claim 8 *	1-7	A 23 L 1/227 A 23 L 1/228 A 23 L 1/229
A	GB-A- 916 284 (AJINOMOTO) * Claims 1-4; page 1, lines 35-45 *	1-7	
A	US-A-3 940 500 (SORTWELL) * Claim 1 *	1	
A	CHEMICAL ABSTRACTS, vol. 76, no. 25, 19th June 1972, page 340, no. 152284c, Columbus, Ohio, US; K. YASUMOTO et al.: "Shiitake flavor. I. New sulfur-containing peptide from Lentinus edodes acting as a precursor for lenthionine" & AGR. BIOL. CHEM. 1971, 35(13), 2059-69	1	
A	CHEMICAL ABSTRACTS, vol. 77, no. 5, 31st July 1972, page 394, no. 32955w, Columbus, Ohio, US; S. SINGH et al.: "Cheese flavor development using direct acidified curd" & J. DAIRY SCI. 1972, 55(6), 744-9	1	
The present search report has been drawn up for all claims			TECHNICAL FIELDS SEARCHED (Int. Cl.4)
Place of search THE HAGUE		Date of completion of the search 12-07-1985	Examiner VAN MOER A.M.J.
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	